



The new two-dimensional light scattering method for recognition of pharmaceutical enantiomers

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ABSTRACT

The results of applying the developed mathematical model of the folding of two-dimensional light scattering (2D-LS) patterns into a descriptor to identify the enantiomers of optically active pharmaceutical substances are presented in the article. The following pharmaceutical substances were a subject of this research: L-valine, D-valine, racemic mixture L, D-valine, L-glucose, D-glucose, and L-ascorbic acid. A digital microscope with high spectral density was used to obtain instant 2D-LS patterns. The obtained data were mathematically processed using the original computer program "Vidan", which uses a mathematical model for the folding of the 2D-LS pattern into descriptors, which are analogs of topological indices in quantitative structure-activity correlations approaches to drug analysis. The 10 descriptors were used as criteria for the difference in the 2D distribution of the scattered light intensity. The use of mono- and multidescrptor analyses allowed us to determine the authenticity of pharmaceutical substances of different classes and their optical isomers. It was found that the dispersion of crystalline substances of optical antipodes up to submicron size led to the leveling of light scattering patterns. The mathematical model was developed and applied for the folding of 2D-LS diagrams into a descriptor. This allowed us to identify the optical antipodes of pharmaceutical substances.

INTRODUCTION

About 40% of finished dosage forms contain chiral pharmaceutical substances, and only a quarter of them are individual enantiomers (Calcatera and D'Acquarica, 2018). As a rule, the pharmacological activity of chiral substances is limited by one of the enantiomers – the eutomer. In some cases, the inactive enantiomer (distomer) can cause unwanted side reactions and even toxicity. The ballast nontoxic enantiomer that undergoes biotransformation exerts an additional burden on the body.

The pharmacy domain that has been actively developed during the last decade is associated with the creation of enantioselective drugs from already known racemic substances (Challener, 2016; McConnell *et al.*, 2007). This so-called "chiral switching" allows the eutomer to be registered as a

new drug. Reducing the development time of a chiral drug largely depends on the efficiency of determining the absolute configuration and the enantiomeric purity of a substance (Calcatera and D'Acquarica, 2018). In scientific research and the pharmaceutical industry, the X-ray crystallography, nuclear magnetic resonance, enantioselective chromatography, vibrational circular dichroism, and polarimetry are used for the recognition and separation of enantiomers (Sangamithra *et al.*, 2019). It was proposed to create the tool kit "Chiral Technology" as a set of methods for determining the absolute stereochemistry and enantioseparation of chiral molecules (McConnell *et al.*, 2007). The need for pure enantiomers requires the selection of the most cost-effective method of analysis, as well as the expansion of the bank of chiral instruments (Challener, 2016). The search for new reliable systems for the recognition of optical isomers is expanding. It should be directed toward methods of identification without destruction and preliminary sample preparation of a pharmaceutical substance.

These criteria are met by the method of two-dimensional dynamic light scattering (2D-LS) using the developed device and original software (Lesnikov *et al.*, 2016).

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The method has successfully proven itself in assessing the quality of liquid dosage forms: suspensions, emulsions, tinctures, and determining the authenticity of medicinal and table waters without opening the package, as well as the stability of cluster structures in waters of different isotopic composition (Bunkin *et al.*, 2018; Goncharuk *et al.*, 2011; Karpov *et al.*, 2011; Koldina *et al.*, 2019; Ul'yantsev *et al.*, 2009). Reliability of measurements, nondestructive nature, low cost, simplicity of calibration procedures, and short measurement times are important advantages of the method in the analysis of pharmaceutical substances.

This work presents the results of applying the method of 2D-LS for the recognition of the antipodes of optically active pharmaceutical substances without preliminary sample preparation.

MATERIALS AND METHODS

The following pharmaceutical substances were a subject of research: L-valine ($\geq 98\%$ for HPLC (High Performance Liquid Chromatography), V0500), D-valine ($\geq 98\%$, 855,987), racemic mixture L, D-valine ($\geq 99.0\%$, 94,640), L-glucose ($\geq 99\%$, G5500), D-glucose ($\geq 99.5\%$, G8270), L-ascorbic acid ($\geq 99.0\%$, A5960). All substances were obtained from Sigma-Aldrich (Saint Louis, MO).

Dispersion of substances was carried out by grinding in a mechanical cutting knife mill (F-306, HT Machinery, Japan) by means of free, direct impact (according to Rumpf) for 20 minutes in a pulse mode (Uspenskaya *et al.*, 2020). The particle size distribution of glucose enantiomers (before and after dispersion) was recorded by low-angle laser light scattering on a Malvern 3,600 particle counter. The radiation source was a low-power helium-neon laser emitting monochromatic light ($\lambda = 633$ nm). The measurements were carried out with stirring by placing the substances in an inert solvent (n-hexane). The distribution of the proportion (%) of particles of a given size from the total number was presented in the range 1–120 μm (Fig. 1).

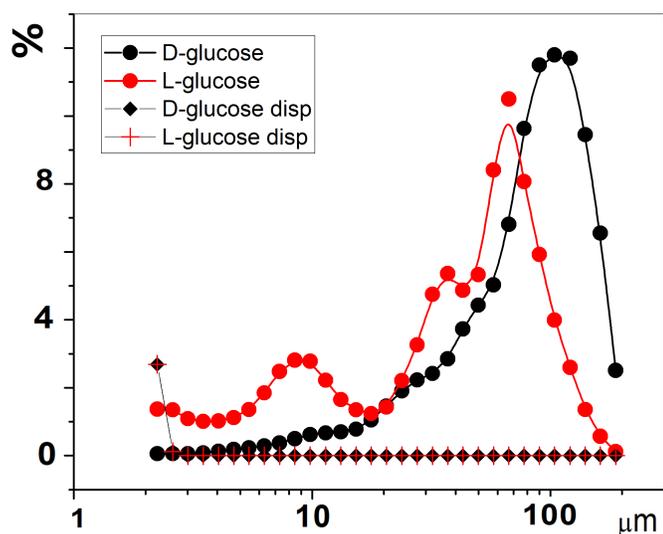


Figure 1. Particle size distribution of glucose optical isomers before and after dispersion ($m = 0.03$ g; $V = 3$ ml; stirring; solvent n-hexane).

The distributions by volume and number fractions (“size spectra”) of D-glucose substance had one maximum at 100 μm , while L-glucose is characterized by the existence of three maxima at 10, 40, and 70 μm . After the dispersion, the particle sizes were in the submicron range.

A digital microscope (Levenhuk DTX 30) with high spectral density LEDs (Light-Emitting Diode) and a video camera connected to a personal computer was used to register instant 2D-LS. The measurements were carried out every second during 120 seconds and were repeated six times ($n = 720$). The data obtained were mathematically processed using the original computer program “Vidan” (Certificate No. 2005612135). The program processes a mathematical model for folding a 2D-LS pattern into descriptors that are analogs of topological indices in the method of quantitative structure-activity correlations (Sprous *et al.*, 2010). The following 10 descriptors were used as criteria for the difference in the 2D distribution of the scattered light intensity: d_1 , d_2 , d_3 , standard deviation (SD)(d_1), SD(d_2), SD(d_3), R_1 , R_2 , R_3 , and r (Koldina *et al.*, 2019). The measurement results made it possible to reveal differences in the nature of the studied samples.

RESULTS AND DISCUSSION

The surface of pharmaceutical substances is a unique characteristic and its properties depend on the chemical nature of the analyzed object and the features of the synthesis. The possibility of describing the frequency and amplitude of surface microvibrations opens prospects for determining the authenticity of a substance up to the assessment of the authenticity of optical antipodes and their racemates.

The integral characteristics of the 2D-LS pattern can be represented in different ways. The primary values of the i -th descriptor on time can be used to calculate the SD and plot the dependence of the average SD of the i -th descriptor on its value $\text{SD}(\bar{d}_i) = F(d_i)$. In addition, it is possible to plot the spectra of relative frequency (RF) of descriptors in coordinates “ $d_i - \text{RF}(d_i)$.” As an example of using the listed mathematical processing methods of light scattering patterns by the first descriptor (d_1), the substances of L-ascorbic acid, L-valine, and L-glucose are considered (Fig. 2). As a consequence, it is easy to identify pharmaceutical substances within the framework of the chosen mathematical model. For the function $\text{RF}(d_1) = F(d_1)$, a set of characteristics such as the position of the maxima, their width, and shape can be used as criteria for authenticity.

The use of mechanochemical treatment of substances in the pharmaceutical industry to increase their biological activity requires new approaches to assessing their quality (Syroeshkin *et al.*, 2019). It was found that the dispersion of enantiomeric substances led to a change in the dynamic light scattering patterns.

Thus, the identification of glucose enantiomers after dispersion becomes impossible since the RF spectra $\text{RF}(d_1) = F(d_1)$ are superimposed on each other. At the same time, native substances occupy separate areas in the range of values of the selected descriptor and can be easily identified (Fig. 3). The absence of differences in the RF of the descriptor for the substances of the D- and L-glucose optical isomers subjected to dispersion correlates with the results obtained using a low-angle laser dispersion meter (see Fig. 1). The absence of particles in the micrometer range is accompanied by the disappearance of

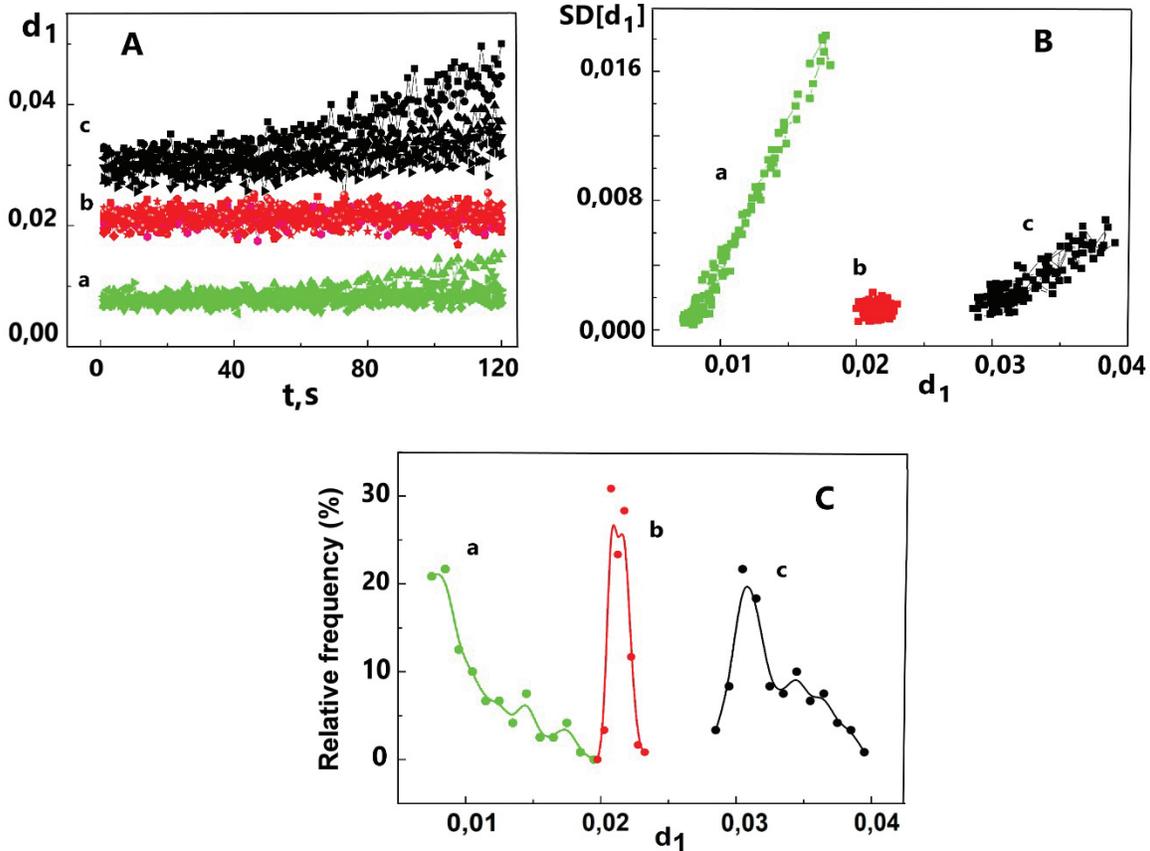


Figure 2. Evaluation of light scattering patterns of pharmaceutical substances in the form of functions: (A) $d_1 = F(t)$; (B) $SD(d_1) = F(d_1)$; (C) $RF(d_1) = F(d_1)$, the relative frequency of the descriptor d_1 . L-ascorbic acid (a), L-valine (b), and L-glucose (c). $n = 720$.

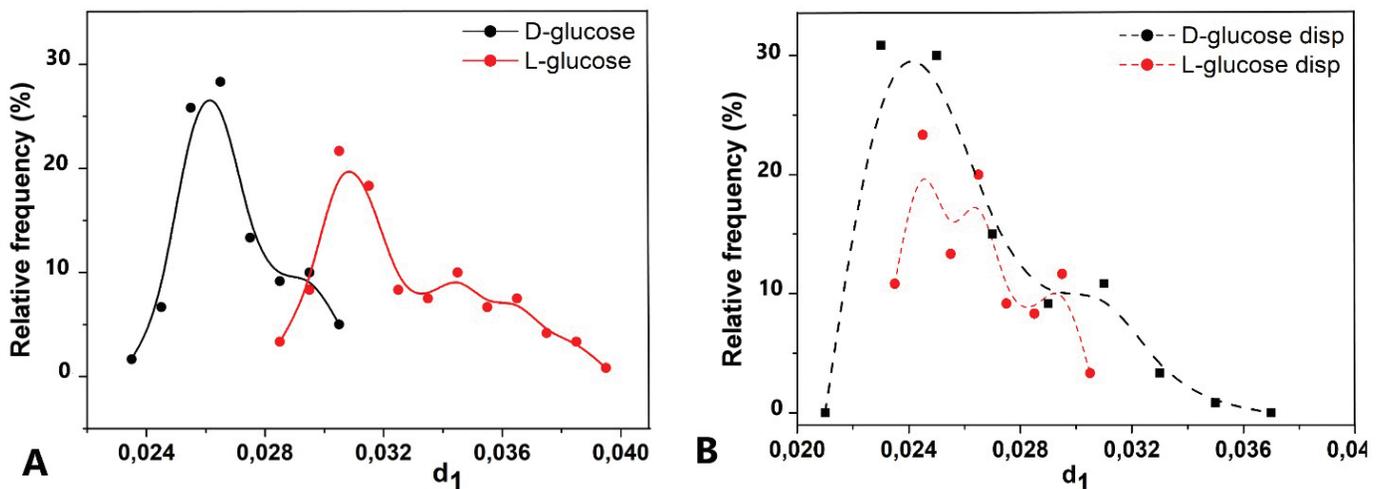


Figure 3. 2D-LS diagrams of glucose enantiomers substances before (A) and after (B) dispersion. $n = 720$.

differences in the characteristics of dynamic light scattering. Thus, light scattering by particles with a diameter of less than 1 micron does not depend on the nature of the substance. Similar patterns were observed for valine (Fig. 4).

Changes in the light scattering on the time of the sample surfaces, described using the descriptors $d_1 = F(t)$ and

$SD(\overline{d_1}) = F(d_1)$, made it possible to identify three substances: racemic valine and its optical antipodes, D-valine, and L-valine. It should be emphasized that the identified substances are chemically indistinguishable, but the peculiarities of their spatial structure are the basis for the observed specificity of dynamic light scattering. All characteristics selected within the

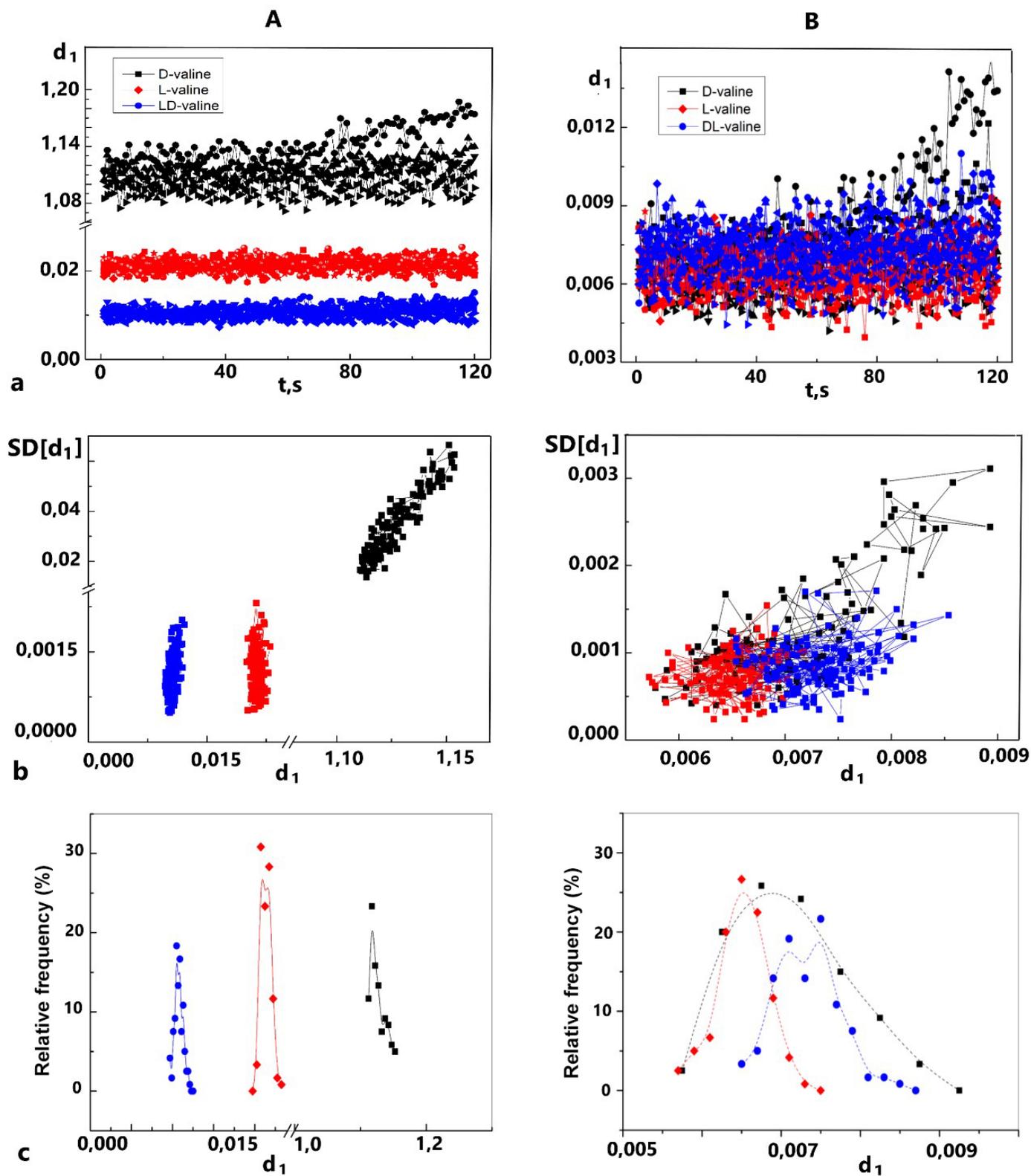


Figure 4. 2D-LS diagrams of the valine optical antipodes and racemic mixture before (A) and after (B) dispersion: a – $d_1 = F(t)$; b – $SD(d_1) = F(d_1)$, c – $RF(d_1) = F(d_1)$, and $n = 720$.

mathematical model framework demonstrated the possibility of identifying both enantiomers and racemate. At the same time, the dispersion of substances led to the leveling of surface

differences, which was reflected in the formation of a common range of the descriptor values d_1 , $SD(d_1)$, and overlapping of the RF bands $RF(d_1)$.

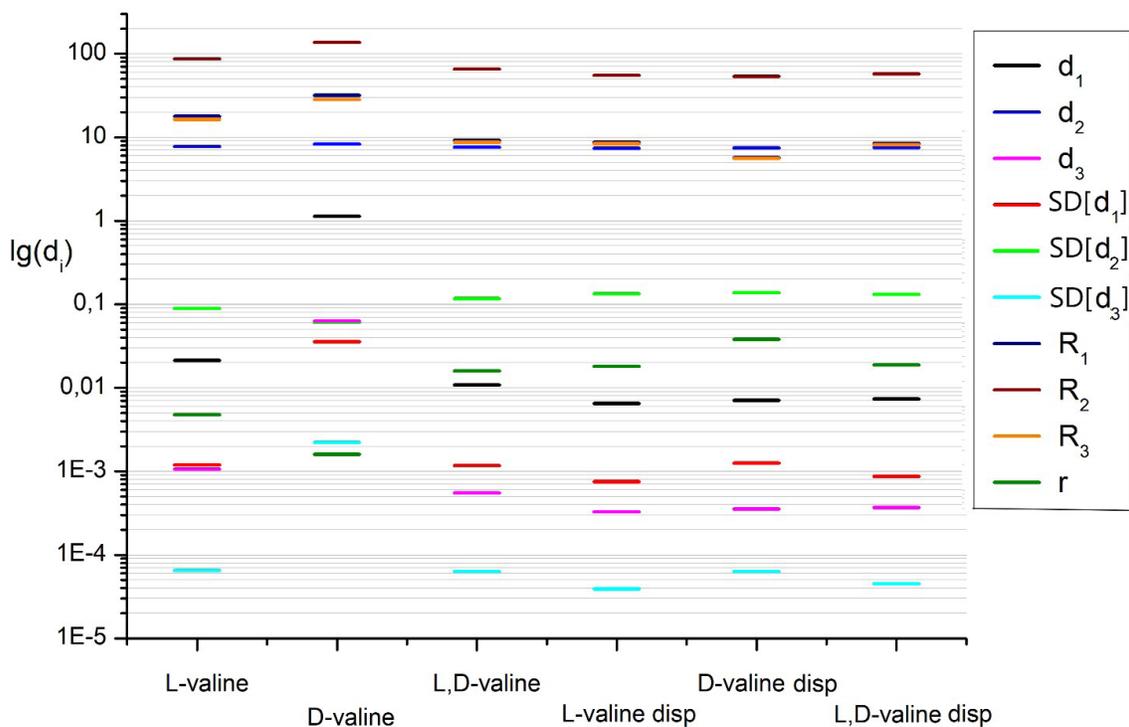


Figure 5. Two-dimensional (2D) diagrams (“fingerprints”) of 10 descriptors of pharmaceutical substances: L-, D-, and L, D-valine.

The research was not limited to the consideration of individual descriptors. For a more detailed analysis, a multidescrptor set was used – a “fingerprint.” It also proved to be specific for the optical antipodes of valine and glucose. This is reflected in the 2D diagram (Fig. 5).

Thus, the light scattering patterns of crystals of amino acid enantiomers have clear topological differences, which make it possible to unambiguously identify the L- and D- isomers and their racemic form. At the same time, the dispersion of crystals of pharmaceutical substances led to a pronounced shift and alignment of the values of all descriptors relative to each other.

Further development of the 2D-LS method opens up prospects for its application for the analysis of the purity and authenticity of optically active and achiral pharmaceutical substances, as well as their finished dosage forms.

CONCLUSION

The use of a mathematical model of folding a 2D-LS pattern into separate descriptors makes it possible to quickly identify compounds that have the same composition and differ in spatial structure. Reliability of measurements, nondestructive nature, low cost, simplicity of calibration procedures, and short measurement times are important advantages of the method in the analysis of pharmaceutical substances, including optical antipodes.

ABBREVIATIONS

2D-LS	Two-dimensional light scattering
RF	Relative frequency

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AUTHORS' CONTRIBUTIONS

Anton V. Syroeshkin is the author of the new method and contributed to the design of the experiment and theoretical interpretation of experimental results. Olga V. Levitskaya played a significant role in conducting the experiment and interpreting the data. Tatiana V. Pleteneva played a leading role in interpreting the data and writing this article. Elena V. Uspenskaya and Maria A. Tribot-Laspierre made significant contributions to the writing and final editing of this article. Irina V. Tarabrina made an active contribution to the acquisition, analysis, and interpretation of data for the article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the publication of this article.

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